

REMARKS/ARGUMENTS

The specification has been amended as supported by the specification and Examples as originally filed. Claims 1-3 have been canceled.

New Claims 4-19 have been added.

New Claims 4-19 are supported by the specification as originally filed and at page 2nd and 3rd full paragraphs.

No new matter is believed to have been added by entry of this amendment. Entry and favorable reconsideration are respectfully requested.

Upon entry of this amendment Claims 4-18 will now be active in this application.

Applicants respectfully request reconsideration of the application, as amended, in view of the following remarks.

The rejection of Claims 1-3 under 35 U.S.C. § 112, 2nd paragraph, is moot in view of the cancellation of these claims.

The present invention as set forth in **new Claim 4** relates to a method for measuring a biodegradation rate of a non-natural organic compound in the presence of a biodegradation medium, comprising:

preparing a sample medium by adding the non-natural organic compound to the biodegradation medium and **without enriching said sample medium with a radioactive carbon isotope ¹⁴C**;

measuring a concentration of ¹⁴C (pMC) of a control medium before biodegradation which is the biodegradation medium without said non-natural compound;

measuring a concentration of ^{14}C (pMC), carbon, and metal as an internal standard of the sample medium before and after biodegradation;

calculating the biodegradation rate by using the obtained pMC values for the following calculation formulae:

$$B = \frac{\left(\text{pMC of control medium} \right) - \left(\text{pMC of sample medium} \right)}{\left(\text{pMC of control medium} \right)} \quad (1)$$

$$C = \frac{\left(\text{pMC of control medium} \right) - \left(\text{pMC of sample medium} \right)}{\left(\text{pMC of control medium} \right)} \quad (2)$$

wherein a carbon content in the sample medium before biodegradation (D) is

$$D = \frac{\text{Carbon content derived from sample (g)}}{B} \quad (3)$$

wherein a carbon content in sample medium after biodegradation (E) is

$$E = D - \text{Amount of carbon dioxide discharged from sample medium (g)} \times \frac{12}{44} \quad (4) \text{ and}$$

wherein a biodegradation rate is

$$\text{Biodegradation rate (\%)} = \left[1 - \frac{E \times C}{D \times B} \right] \times 100 \quad (5)$$

$$= \left[1 - \frac{E \times C}{\text{Carbon content derived from sample (g)}} \right] \times 100 \quad (6);$$

or

wherein said biodegradation rate is determined using the following calculation formulae

$$H = \frac{F}{G} \times D \quad (7)$$

$$E = \frac{J}{I} \times K = \frac{J}{I} \times \frac{F}{G} \times D \quad (8)$$

wherein

F: metal concentration of sample medium before biodegradation;

G: carbon concentration of sample medium before biodegradation;

I: metal concentration of sample medium after biodegradation;

J: carbon concentration of sample medium after biodegradation;

D: carbon content (g) of sample medium before biodegradation;

E: carbon content (g) of sample medium after biodegradation;

H: metal content (g) of sample medium before biodegradation;

K: metal content (g) of sample medium after biodegradation; and K = H.

New Claim 16 relates to a method for measuring a biodegradation rate of a non-natural organic compound in the presence of a biodegradation medium, comprising:

mixing a non-natural organic compound and a natural substance containing a radioactive carbon isotope ^{14}C having a function of causing biodegradation of said non-natural organic compound, to obtain a sample **without enriching said sample with a radioactive carbon isotope ^{14}C** ;

charging said sample into a reaction tank of a biodegradation reaction apparatus;
carrying out a biodegradation reaction of said sample at a predetermined temperature
and for a predetermined time while an air having carbon dioxide completely removed
therefrom is introduced into a vicinity of a bottom of the reaction tank;

wherein said air is introduced into said reaction tank through a carbon
dioxide absorption tank, to inhibit carbon dioxide from the
atmosphere to enter into said reaction tank;

after completion of said biodegradation reaction, burning a medium containing said
non-natural organic compound of the biodegraded sample, in air having carbon dioxide
removed therefrom or in pure oxygen, to convert the medium into carbon dioxide;

passing said carbon dioxide through a carbon dioxide absorption tank, and absorbing
and trapping said carbon dioxide in said carbon dioxide absorption tank;

measuring a content (pMC) of a radioactive carbon isotope ^{14}C in said carbon dioxide;
and further comprising:

determining pMC of said medium containing said sample obtained as described above
before and after biodegradation;

determining pMC of a control medium of a radioactive carbon isotope ^{14}C obtained in
the same manner by using a medium containing a natural substance alone without addition of
the non-natural organic compound; and

calculating the biodegradation rate by using the obtained pMC values for the
following calculation formulae:

$$B = \frac{\left(\begin{array}{c} \text{pMC of control medium} \\ \text{before biodegradation} \end{array} \right) - \left(\begin{array}{c} \text{pMC of sample medium} \\ \text{before biodegradation} \end{array} \right)}{\left(\begin{array}{c} \text{pMC of control medium} \\ \text{before biodegradation} \end{array} \right)} \quad (1)$$

$$C = \frac{\left(\text{pMC of control medium} \right) - \left(\text{pMC of sample medium} \right)}{\left(\text{pMC of control medium} \right)} \quad (2)$$

wherein the pMC of the control medium does not change before and after biodegradation;

wherein a carbon content in sample medium before biodegradation (D) is

$$D = \frac{\text{Carbon content derived from sample (g)}}{B} \quad (3)$$

wherein a carbon content in sample medium after biodegradation (E) is

$$E = D - \text{Amount of carbon dioxide discharged from sample medium (g)} \times \frac{12}{44} \quad (4) \text{ and}$$

wherein a biodegradation rate is

$$\text{Biodegradation rate (\%)} = \left[1 - \frac{E \times C}{D \times B} \right] \times 100 \quad (5)$$

$$= \left[1 - \frac{E \times C}{\text{Carbon content derived from sample (g)}} \right] \times 100 \quad (6).$$

New Claim 17 relates to a method according to claim 16, wherein said medium subjected to biodegradation comprises a metal as an internal standard which does not biodegrade;

wherein a total content of the metal is constant before and after the biodegradation operation; and

wherein a biodegradation rate is determined using the following calculation formulae

$$H = \frac{F}{G} \times D \quad (7)$$

$$E = \frac{J}{I} \times K = \frac{J}{I} \times \frac{F}{G} \times D \quad (8)$$

wherein

F: metal concentration of sample medium before biodegradation;

G: carbon concentration of sample medium before biodegradation;

I: metal concentration of sample medium after biodegradation;

J: carbon concentration of sample medium after biodegradation;

D: carbon content (g) of sample medium before biodegradation;

E: carbon content (g) of sample medium after biodegradation;

H: metal content (g) of sample medium before biodegradation;

K: metal content (g) of sample medium after biodegradation; and K = H.

None of Larson, Melvin and Knowles discloses or suggests a method for measuring a biodegradation rate of a non-natural organic compound sample without enriching the sample with radioactive carbon isotope.

None of Larson, Melvin and Knowles discloses or suggests to use a metal as an internal standard.

In **new Claim 4**, biodegradation rate is calculated by measuring the concentration of ^{14}C , carbon and metal of the sample medium before and after biodegradation.

The metal (as an internal standard) does not biodegrade and does not evaporate. Therefore a total content of the metal hardly changes before and after biodegradation.

A value so-called percent Modern Carbon (pMC) is calculated based on a concentration of ^{14}C in atmosphere as of 1950 as 100 % under an international agreement. This is described in, for example, Currie, L. (2004). "The Remarkable Metrological History of Radiocarbon Dating II". *J. Res. Natl. Inst. Stand. Technol* 109: 185-217. The reference is submitted via Information Disclosure Statement.

The pMC at 2002 falls within a range of 110 to 111 %. On the other hand the concentration of ^{14}C of the compost of Example 1 of the present application is a value of 115.82% which is higher than the above range. The reason behind it is that the concentration of ^{14}C of compost reflects the pMC of before 2002. That is to say, the concentration of ^{14}C in the atmosphere has been increased by many nuclear tests in 1960s, and it has been diluted with carbon dioxide gas from fossil fuel etc. in the course of industrial development after 1960s. These facts are described in the above literature.

In Example 1 of the present application, one can obtain the biodegradation rate by substituting values of Table 1 in the formulae (1), (2), (3), (7), and (8), obtaining values of symbols B, C, D and E, and substituting these values in the formulae (5). See pages 7-9 and 12 of the specification.

Thus, new Claims 4-15 should not be rejected under 35 U.S.C. § 112, 2nd paragraph.

The rejections of Claims 1-3 over Larson, Melvin and Knowles are moot in view of the cancellation of these claims.

Regarding new Claim 4, the biodegradation rate may be measured without enriching the sample medium with radioactive carbon isotope ^{14}C . It is one advantage of the present invention.

Larson describes a method for measuring the biodegradation rate of an unnatural compound by measuring the concentration of radioactive carbon isotope ^{14}C in a biodegradation medium and measuring the biodegradation rate of unnatural organic compound from the difference in the measured ^{14}C concentration and ^{14}C concentration in the present carbon. However, Larson enriches the sample medium with radioactive carbon isotope ^{14}C .

The following expressions are at lines 21-24 of page 160 of Larson.

“Labeled and unlabeled OTAC were synthesized in our laboratories Chemical and radiochemical purity of ^{14}C - OTAC were both greater than”

These words “labeled” and “radiochemical” mean that the sample mediums are enriched with radioactive carbon isotope ^{14}C . Also “*C” in the Table 1 of page 161 means that the carbon is enriched with radioactive carbon isotope ^{14}C .

Larson enriches the sample medium with ^{14}C , biodegrades the sample, collects a carbon dioxide produced, determines the ^{14}C content of the collected fraction and thus obtains the biodegradation rate of the sample.

In adopting Larson’s method, a person of ordinary skill in the art has to add a great amount of ^{14}C to the sample medium, enough to neglect the ^{14}C derived from the biodegradation medium.

Larson’s method has problems in that it needs a lot of expensive radioactive carbon isotope ^{14}C and a special measures against radiation and the method has measurement errors.

The inventors of the present invention focused on a very small amount of ^{14}C in a biodegradation medium and completed the present invention. A non-natural organic compound produced from a fossil fuel such as a crude oil does not contain ^{14}C . On the other

hand a natural substance such as compost, active sludge, or soil suspension contains a very small amount of ^{14}C and is capable of biodegrading an organic compound and working as a biodegradation medium. When a natural substance is used as a biodegradation medium and a sample medium containing a non-natural organic compound and a biodegradation medium is biodegraded, carbon dioxide is generated. The inventors of the present invention have found that the biodegradation rate can be measured from a content of ^{14}C in the carbon dioxide.

Larson neither discloses nor suggests a method for measuring a biodegradation rate of a non-natural organic compound sample without enriching the sample with radioactive carbon isotope ^{14}C . Therefore the present invention of new Claims 4 and 16 are not anticipated by Larson.

The present invention of new Claims 4 and 5 uses a metal as an internal standard. In the present invention, the metal does not biodegrade or evaporate even in a biodegradation operation and a total content of the metal hardly changes before and after the biodegradation.

Melvin discloses a method for determining an organic compound with a liquid chromatogram by using DMP as an internal standard after chemical degradation of ammonia.

Applicants note the following about Melvin. First, the degradation of ammonia is not a biodegradation but a chemical degradation. Second, the internal standard does not exist during the degradation, but is used after the degradation. Third, the internal standard is not a metal but an organic compound.

Knowles et al neither disclose nor suggest a metal as an internal standard but only a biodegradation of metal cyanides.

None of Larson, Melvin and Knowles discloses or suggests a method for measuring a biodegradation rate of a non-natural organic compound sample without enriching the sample with radioactive carbon isotope

None of Larson, Melvin and Knowles discloses or suggests to use a metal as an internal standard.

Thus, Claims 4 and 16 should not be rejected over Larson, Melvin and Knowles.

In regard to the Examiner's request to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made, Applicants have confirmed that all claims were commonly owned at the time the present invention was made.

This application presents allowable subject matter, and the Examiner is kindly requested to pass it to issue. Should the Examiner have any questions regarding the claims or otherwise wish to discuss this case, he is kindly invited to contact Applicants' below-signed representative, who would be happy to provide any assistance deemed necessary in speeding this application to allowance.

Respectfully submitted,

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